Synthesis, conformational studies and anthelmintic activity of a constrained PF1022A analogue

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Abstract: A mono-proline analogue of the potent anthelmintic cyclooctadepsipeptide PF1022A has been synthesized. NMR studies in combination with molecular dynamics simulations demonstrate that the confirmation of this analogue is both rigidified and distorted compared with the asymmetric conformation of PF1022A. The backbone conformation is significant with respect to anthelmintic activity.

Keywords: PF1022A; anthelmintic; mono proline analogue; bioactivity

1 INTRODUCTION

Parasitic nematodes are a major cause of morbidity and mortality in man and also cause widespread loss of food production by infection of livestock.[1] A milestone in the chemotherapy of nematode infections, especially in animals, was the discovery of the milbemycins[2,3] and the avermectins[4] during the 1970s. Since the discovery of these highly active macrolides, reports of potent new classes of anthelmintics have been scarce.[5–7] One of the most outstanding anthelmintics, recently reported, is the cyclooctadepsipeptide PF1022A, the most active member of a new class of anthelmintic agents (Fig 1).[8] PF1022A exhibits a potent and rapid anthelmintic action *in vitro*, suggesting that the compound acts as a neurotoxin in nematodes.[9–11] Following oral or intravenous application PF1022A shows efficacious in-vivo activity against economically relevant nematodes at dosages comparable with most commercially available anthelmintics. However, problems arise from the lipophilicity of PF1022A resulting in insufficient parenteral delivery for some nematode species.[9] Nevertheless the excellent intrinsic anthelmintic activity and its unique mode of action make PF1022A a promising new lead structure.

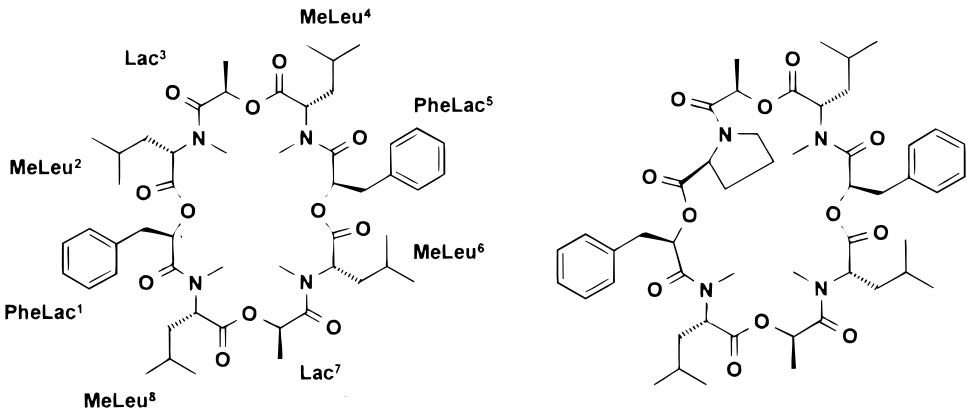

Figure 1. Structure of PF1022A and the mono-proline analogue (**2**).

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(*Received 19 March 1998; revised version received 28 August 1998; accepted 13 November 1998*)

 Pestic Sci 0031–613X/99/$17.50

The crystal structure of PF1022A has recently been determined by single-crystal X-ray analysis.[12] Despite the occurrence of different space groups, structures determined from crystals obtained from methanol (monoclinic, P 2_1), or acetone (orthorhombic, P $2_12_12_1$) were nearly identical. Both showed one *cis*-amide bond between MeLeu[2] and Lac[3], conferring asymmetry on the molecule. However, in solution in chloroform, PF1022A exists as a 3 : 1 mixture of two conformers which interconvert very slowly on the NMR time-scale. The main isomer corresponds to an asymmetric conformer, while the minor isomer can be assigned to a symmetric conformer with all four amide bonds in the *trans*-configuration (Fig 2).

As the first step in identifying the pharmacophore within PF1022A, we were interested in the significance of the two PF1022A conformers. With the aim of stabilizing the asymmetric conformer, we exchanged one leucine for a proline residue, the latter being well-known for its stabilizing effect on *cis*-amide bonds.

2 MATERIALS AND METHODS

2.1 Synthesis

Several total syntheses of PF1022A using chemistry in solution[13–16] and on solid phase[17] have been accomplished to date. The synthesis of the proline analogue, (Fig 1; **2**) was based on methodology developed during our total synthesis of PF1022A[13] (Fig 3), employing *tert*-butyl esters and *N*-benzyl groups for the orthogonal protecting group strategy. The starting materials (*S*)-2-chloropropanoic acid,[18] (*S*)-2-chloro-3-phenylpropanoic acid,[18] and *N*-methyl-*N*-benzylleucine[19,20] were prepared following standard literature procedures. (*L*)-Proline was benzylated with benzyl bromide under phase-transfer catalysis as described by Belokon *et al.*[21] The hydrogenolytic debenzylation of the *N*-benzylproline containing building blocks proved to be somewhat critical with respect to racemization. Best results were obtained via a two-step procedure, using 1-chlororethyl chloroformate as the debenzylation reagent and subsequent cleavage of the intermediate chloroethyl urethane with methanol.[22,23] The macrocyclization was accomplished between the *N*-terminal leucine and the *C*-terminal phenyl lactic acid residues in 55% yield.

Characteristic [^{1}H]NMR (500 MHz, deuterochloroform) signals: $\delta = 3.5$ (dd, 2H), 4.63 (q, 1H, H_α-Lac), 4.72 (dd, 1H, H_α-Pro), 5.33–5.45 (m, H_α-Leu), 5.49 (t, 1H, H_α-PheLac), 5.60 (m, 2H, H_α-Lac + H_α-PheLac).

FAB MS: m/z (%) = 919 (28, $(M + H)^+$).

2.2 Biological testing

For all experiments, male mice of the strain SPF/CFW1 with 16–18 g body weight at the start of the experiment were used. Groups of five animals, housed in Makrolon cages were given water and 'Sniff' rat feed, 13-cm pellets, *ad libitum*. Mice were infected with a mixed culture of the nematodes *Nematospiroides dubius* (=*Heligmosomoides polygyrus*), Dujardin, *Heterakis spumosa*, Schneider and *Trichinella spiralis* Owen. Third-stage larvae of *N dubius* were collected from mouse faeces 21 days post-infection (pi), *H spumosa* eggs were obtained from female worms isolated from the mouse colon 35–42 days pi and were then incubated at 27°C for three weeks. *T spiralis* larvae were obtained from pepsin-treated skeletal muscles and diaphragms of WistarW64 rats 20 days pi. Test compounds (1 g) were dissolved or suspended in the emulsifier 'Cremophor' El (0.2 ml) and 0.5 ml of the solution or suspension was administered per 20 g of mouse once daily for four consecutive days. One mouse received the highest dosage of 100 mg kg^{-1}; if anthelmintic activity was observed at this dosage, lower dosages of 50, 25, 10 and 5 mg kg^{-1} were tested until anthelmintic activity could no longer be detected. The mixed-parasite infection was introduced into the mice in a stepwise manner. They were first infected orally with 90 embryonated *H spumosa* eggs, fol-

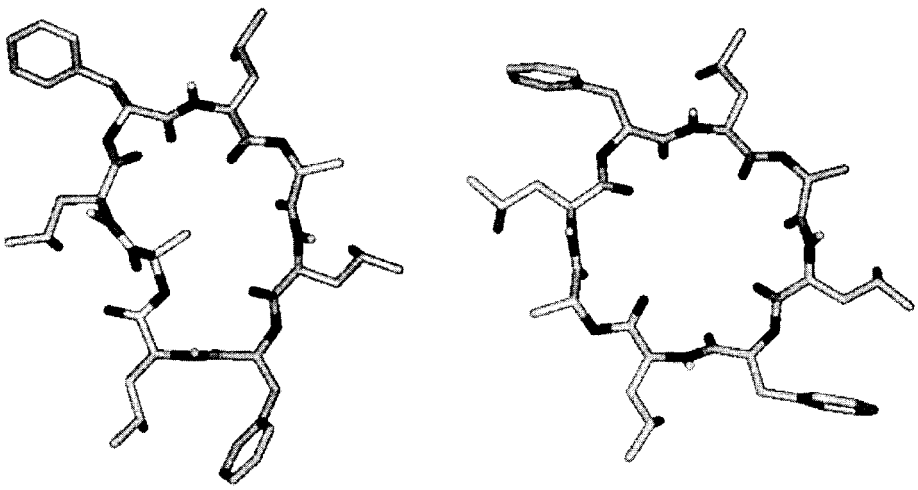

Figure 2. Structure of the asymmetric and symmetric PF1022A conformers.

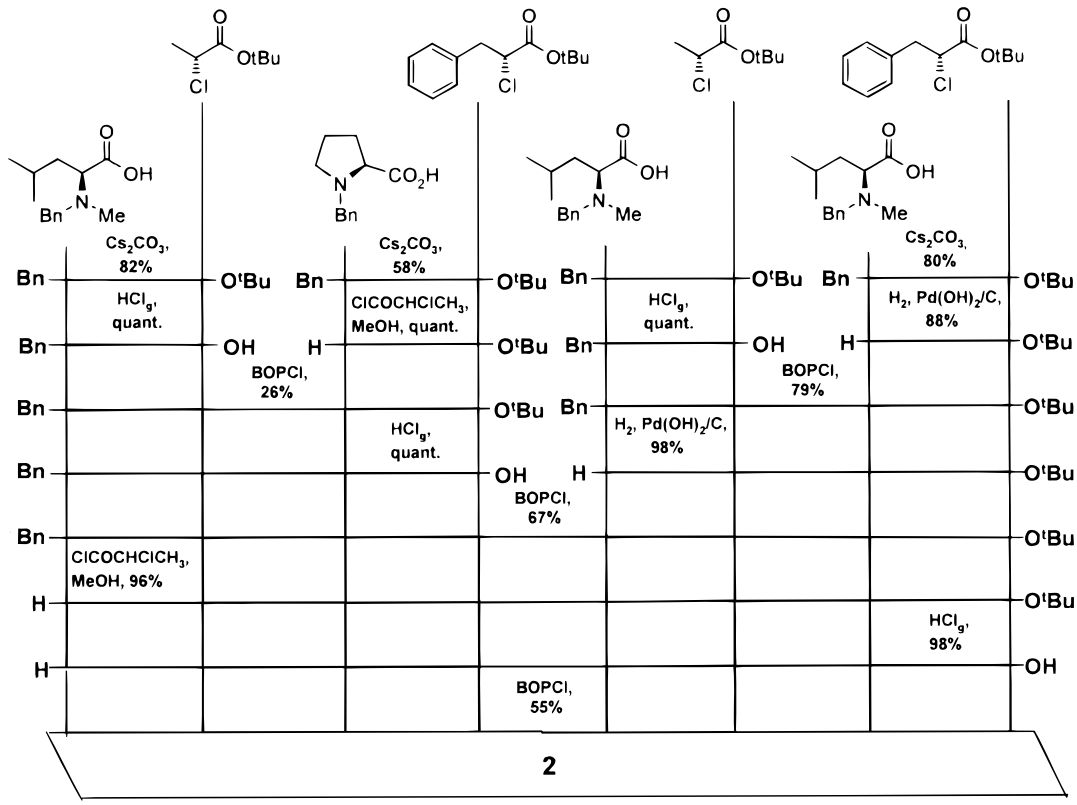

Figure 3. Synthetic scheme for the mono-proline analogue.

lowed seven days later with 100 *T spiralis* larvae; 27 days later, 60–70 larvae of *N dubius* were introduced. Treatment began on day 46 pi and ended on day 49 pi. After a further eight days the animals were killed with carbon dioxide and then dissected. *H spumosa* were isolated from the caecum and the colon, numbers being determined by microscopy. Removal of the duodenum and treatment of this organ in a compressor, followed by microscopic examination (magnification × 40), allowed the numbers of *N dubius* remaining to be determined. The numbers of *T spiralis* present in 1 cm^2 of abdominal muscle, removed by dissection and compressed between two plastic sheets using a hand press, were determined by examination under a binocular microscope (magnification × 40). Activity against the three nematodes was evaluated on a single scale 0–3 where 3 represented cure (no parasites detectable), 2 effective (<20% of parasites remaining), 1 trace effect (<50% of parasites remaining) and 0 ineffective (>50% of parasites remaining).

Sheep (Merino or Schwarzkopf breed, 25–35 kg body weight) were infected experimentally with 5000 *Haemonchus contortus* Rud L3 larvae and treated with the test substance after the end of the prepatency period of the parasite. The test compounds were administered orally in gelatine capsules or intravenously, as recently described.[24] Anthelmintic effects of the test substances were measured as a function of the reduction in the sheep faecal egg count. For the purpose of counting eggs, freshly obtained faeces from experimental animals were prepared using the McMaster method as modified by Wetzel and the egg count was calculated per gram of faeces.[25] The egg counts were determined at regular intervals before and after treatment.

3 RESULTS AND DISCUSSION

The proton NMR spectrum of **2** (in deuterochloroform) shows only one conformer, containing a *cis*-amide bond between Lac^3 and Pro^2. Furthermore, in the NOESY spectrum, a NOE between the β-hydrogens of Lac^3 and the α-hydrogen of Lac^7 is observed which demonstrates a significant difference in the backbone conformation of **2** compared with the asymmetric conformation of PF1022A. This transannular proton–proton contact suggests a ring perturbation in the area of $MeLeu^6$–Lac^7–$MeLeu^8$ in such a way that Lac^3 and Lac^7 are forced together (Fig 4). According to molecular dynamics simulations, the distorted conformation of **2** can also be adopted by the PF1022A molecule, but is energetically disfavoured.

Interestingly, **2**, which is slightly less lipophilic ($\log P = 5.2$) than PF1022A ($\log P = 5.9$), exhibited a remarkably reduced anthelmintic activity in sheep at a dosage of 0.5 mg kg^{-1}. In comparison, PF1022A gave full control of *H contortus* at a dose of 0.1 mg kg^{-1} when administered intravenously (Table 1). According to these results, compound **2** was ineffective against *N dubius* and *H spumosa* in mice even at a dosage of 100 mg kg^{-1} (Table 2). We suggest

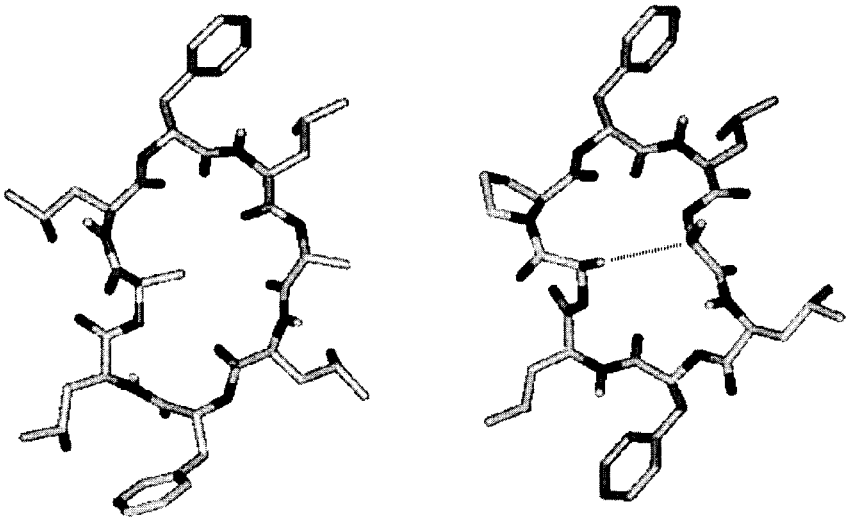

Figure 4. Comparison of the asymmetric conformation of PF1022A and **2**.

Table 1. Effect of intravenous treatment of sheep with PF1022A and its proline analogue (**2**) level of *Haemonchus contortus* eggs in faeces

	Dosage ($mg\,kg^{-1}$)	*Eggs per g of faeces before treatment*	*Eggs per g of faeces after treatment* ($\pm SE$)	*Reduction of eggs per g of faeces after treatment* (%)
Control[a]	0	6667 ($n = 2$)	6142 (± 364) ($n = 8$)[a]	7.9
2	0.5	3533 ($n = 2$)	1693 (± 291) ($n = 10$)	52.1
PF1022A	0.1	1833 ($n = 2$)	0 ($n = 3$)	100
PF1022A	0.01	1000 ($n = 2$)	573 (± 182) ($n = 5$)	42.7

[a] Controls were not treated with any compound; egg counting was undertaken at the corresponding times compared to treated animals.

Table 2. Anthelmintic activity of PF1022A and its proline analogue, **2**, against different nematodes in experimentally infected mice

Compound	*Dosage* ($mg\,kg^{-1}$)	*Activity*[a] *against*		
		Nematospiroides dubius	*Heterakis spumosa*	*Trichinella spiralis*
20PF1022A	100	3[a]	3	0
	50	3	3	0
	25	2	0	0
	10	1	0	0
	5	0	0	0
2	100	0	0	0

[a] See Section 2.2; each dose level was tested in two different experiments with three mice each.

that the reason for the loss of anthelmintic activity is the perturbation of the depsipeptide backbone.

In summary, the first spectroscopic and biological data pertaining to a rigidified PF1022A analogue clearly demonstrate that the backbone conformation of the molecule is significant with respect to anthelmintic activity.

ACKNOWLEDGEMENTS

We thank Meiji Seika Kaisha Ltd for providing us with an authentic sample of PF1022A.

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